

MERCURY AND SELENIUM CONCENTRATIONS IN LARGEMOUTH BASS AND OTHER FISHES OF THE LOWER SUWANNEE NATIONAL WILDLIFE REFUGE

PUBLICATION NO. PCFO-EC 92-01

U.S. FISH AND WILDLIFE SERVICE FISH AND WILDLIFE ENHANCEMENT PANAMA CITY FIELD OFFICE 1612 JUNE AVENUE PANAMA CITY, FLORIDA 32405

MICHAEL S. BRIM ENVIRONMENTAL CONTAMINANTS SPECIALIST

DIANE BATEMAN
ASSISTANT ENVIRONMENTAL CONTAMINANTS SPECIALIST

ROBERT JARVIS BIOLOGICAL AID

GAIL A. CARMODY PROJECT LEADER

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ABSTRACT:

From March 22 to April 7, 1990, 71 largemouth bass (*Micropterus salmoides*) and four other fish species (total of seven fish) were collected from ten locations on or adjacent to the Lower Suwannee National Wildlife Refuge and in the Santa Fe River, Florida. The fish were collected for mercury and selenium analyses of muscle tissue. Fifty-five percent of the bass had mercury levels that exceeded the Florida limited-consumption concentration of 0.5 parts per million (ppm), wet weight. Three percent exceeded Florida's no-consumption concentration of 1.5 ppm. Two other species, spotted gar (*Lepisosteus oculatus*) and yellow bullhead (*Ameiurus natalis*), also had high levels of mercury.

Evaluation of weight, length and age of largemouth bass did not provide mechanisms by which recreational fishermen could selectively retain bass that are low in mercury. No direct association was observed between mercury and selenium concentrations in muscle tissue. However, fish egg data (from this study and from various other sources) indicate that selenium concentrations greatly exceed mercury concentrations.

Four sampling locations, the Suwannee River, Santa Fe River, Sand Fly Creek and Week Creek appear to provide high mercury environments for largemouth bass. Fish and wildlife trust resources may be at some risk when utilizing these areas. Additional geographic and biotic environmental contaminant work is recommended.

KEY WORDS:

Mercury, selenium, largemouth bass, Suwannee bass, yellow bullhead, channel catfish, spotted gar, fish eggs, Suwannee River.

PREFACE

This report is written for the Fish and Wildlife Refuge System. However, we realize that much of the information contained in the report may be passed on to the general public by the Refuge Manager. Therefore, the report is prepared in a "non-technical" format. English measurements are used throughout. We hope the format provides information for Refuge personnel and recreational fishermen that can be easily and immediately understood. Metric measurements for the database (and their conversions to English units) can be found in Appendix H. In addition, we have used wet weight (fresh weight) values for mercury and selenium concentrations in all discussions about those metals. Wet weight values are consistently used by the State of Florida in setting consumption advisories. Sample dry weight values and tissue moisture values are presented in Appendix H.

CONTENTS

Page
ACTi
CE
F TABLESiv
F FIGURES
F APPENDICES
OWLEDGMENTSvii
DUCTION
JRY
UM
ACTIONS OF MERCURY AND SELENIUM IN BIOTA
ESCRIPTION
ING STATIONS
RIALS AND METHODS
TS
SSION
USIONS AND RECOMMENDATIONS
TURE CITED 25
TURE CITED

TABLES

Num	nber Pag	е
1	Fish collections at locations in or adjacent to the Lower Suwannee NWR, 1990	8
2	Comparison of mean mercury and selenium concentrations between largemouth bass age groups	6
3	Age, and mercury and selenium content of largemouth bass collected at the Lower Suwannee NWR; and the approximate percentage of each waterbody's drainage basin located inside the Refuge boundaries. Averages are arithmetic/ geometric means	0
4	Age, and mercury and selenium content, of age group 4 largemouth bass collected at the Lower Suwannee NWR. Also, the approximate percentage of each waterbody's drainage basin located inside the Refuge boundaries. Concentrations are expressed in ppm, wet weight Averages are arithmetic/ geometric means	0
5	Comparison of mercury and selenium concentrations in fish eggs from the Lower Suwannee NWR and other collection locations	2
6	Partitioning of mercury in some body compartments of two fish species. Fish were collected in St. Andrew Bay, Florida (spotted sea trout), Apalachicola River, Florida, and Flint River, Georgia (striped bass). Mercury values are ppm wet weight. USFWS data, Panama City, Florida	4

FIGURES

Numl	ber experience of the second s	Pa	ige
1	Location of Lower Suwannee National Wildlife Refuge	. 	. 4
2	Suwannee River - Fish Collection Stations March/April 1990		. 6
3	Variation of mercury concentration in largemouth bass by weight class groups. Lower Suwannee NWR, 1990		10
4	Variation of mercury concentration in largemouth bass by weight in ounces. Lower Suwannee NWR, 1990		10
5	Variation of mercury concentration in largemouth bass by length groups. Lower Suwannee NWR, 1990		11
6	Variation of mercury concentration in largemouth bass by age. Lower Suwannee NWR, 1990		11
7	Variation of selenium concentration in largemouth bass by pound class. Lower Suwannee NWR, 1990		13
8	Variation of selenium concentration in largemouth bass by weight in ounces. Lower Suwannee NWR, 1990		13
9	Variation of selenium concentration in largemouth bass by inch class. Lower Suwannee NWR, 1990		15
10	Variation of selenium concentration in largemouth bass age groups. Lower Suwannee NWR, 1990		15
11	Mean mercury concentrations of largemouth bass by age group		18
12	Mean selenium concentration of largemouth bass by age group		18
13	Mercury concentration in muscle tissue of largemouth bass, Lower Suwannee NWR and Santa Fe River, 1990		19
14	Selenium concentration in muscle tissue of largemouth bass, Lower Suwannee NWR and Santa Fe River, 1990		19

LIST OF APPENDICES

Apper	ndix	Page
Α	The Nature of Mercury	. 27
В	The Nature of Selenium	. 30
С	Interactions of Mercury and Selenium in Biota	. 32
D	Mercury and Selenium Concentrations in Largemouth Bass Muscle Tissue. Fish are grouped by age and sex. Tables D.1; D.2; D.3; D.4	. 33
E	Lower Suwannee National Wildlife Refuge Study Site Locations	. 38
F	Standard Operating Procedure for Collection of Fish Tissue Samples	. 39
G	Laboratory QA/QC for Mercury and Selenium Analyses	. 41
Н	Printout of Study Data	. 50

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We were assisted in report preparation and review by Drs. Don Schultz and Charles Facemire, Region 4 Environmental Contaminants Coordinators; Gail Carmody, Panama City Field Office Project Leader; Lorna Patrick, Panama City Field Office, Enhancement Division; and Chuck Reynolds, Panama City Field Office, Fisheries Assistance.

INTRODUCTION

In September 1982, the Florida Game and Fresh Water Fish Commission began a survey of fishes in the Chipola River of northwest Florida to determine if contamination of fish had occurred. This action was prompted by pollution within the drainage basin of the Chipola River from a battery salvage plant located in Jackson County. Largemouth bass (Micropterus salmoides) collected from the Dead Lakes area of the Chipola River did have elevated mercury levels. "natural" background measurements for comparison, the "pristine" Santa Fe River was also chosen for sampling. Results were surprising. Elevated mercury levels were detected in Santa Fe River bass. These results led to the formation of an informal interagency task force composed of personnel from the Florida Game and Fresh Water Fish Commission, the Florida Department of Environmental Regulation, and the Florida Department of Health and Rehabilitative Services. Subsequently, a systematic statewide mercury investigation was initiated that involved the sampling of about 20 Florida lakes or streams each year. In 1988, the investigation revealed a mercury problem in largemouth bass and other species collected in the Everglades waterways of south Florida.

As a result of the State's mercury investigation, fish consumption health advisories were formulated by the Department of Health and Rehabilitative Services (HRS) (1989) for largemouth bass and other species. The advisories recommend that when average concentrations of mercury (in the edible portion, i.e., fillet) are between 0.5 ppm and 1.5 ppm wet weight, adults should limit their consumption to no more than one meal of fish per week. Nursing mothers, women who are pregnant or anticipate bearing children, and children under 15 years of age are advised not to eat these fish more than once a month. Fish that contain more than 1.5 ppm of mercury should not be eaten by anyone (HRS, 1989). Approximately one million acres of the Everglades and another one million acres of Florida freshwater areas have been posted with advisories (Lambou, et al., 1991).

Because of the large area of National Wildlife Refuge (NWR) lands in Florida not previously included in the State investigation, the U.S. Fish and Wildlife Service (Service) sampled selected refuges in Florida to determine mercury levels in the fish within these refuges. Many federal trust resource species utilize the Florida refuges, including endangered species, migratory birds, and anadromous fishes.

The objectives of the Lower Suwannee NWR study were to determine if fish had levels of contamination that would trigger a consumption advisory and which might also significantly affect individuals or populations of fish and wildlife resources under refuge management. The present investigation involved the sampling of upper

SITE DESCRIPTION

The Lower Suwannee NWR (Figure 1) was established in 1979 under the Fish and Wildlife Act. The Refuge encompasses 50,000 acres on both sides of the Suwannee River in Levy and Dixie Counties, Florida. When anticipated future acquisition is complete, the Refuge will include over 55,000 acres. This acquisition will ensure that a majority of the river-delta estuarine system of the Suwannee River will lie within the Refuge.

The Refuge was acquired to provide endangered and threatened species, other trust species, and resident species of fish and wildlife with desirable habitat conditions, and protection when necessary. The Refuge includes 26 miles of waterfront on the Gulf of Mexico and encompasses a rich diversity of wildlife habitats. Habitats include tidal estuarine marshes, floodplain bottomland hardwood forests, cypresslined creeks, sloughs, wooded swamps and upland areas forested with oak, pines and other species. Approximately 50 miles of the Suwannee River and associated creeks and sloughs, and another 50 miles of tidal creeks are contained within the Refuge. These diverse habitats comprise one of the largest undeveloped river delta/estuarine complexes in the United States.

As a result of the habitat diversity, wildlife resources at the Lower Suwannee NWR are broad and varied. Marine mammals, such as the endangered West Indian manatee (*Trichechus manatus latirostris*) and the protected bottle-nosed dolphin (*Tursiops truncatus*), along with several species of endangered marine turtles, frequent the coastal waters of Suwannee Sound. The Sound receives a constant influx of nutrients from the river system, and has numerous offshore islands and tidal flats which provide excellent coastal habitat.

Over 250 species of migratory birds have been identified utilizing the Refuge. Osprey (*Pandion haliaetus*), American swallowtail kite (*Elanoides forficatus*), and many species of wading birds feed, rest, and nest on the Refuge. Natural salt marshes and tidal flats attract thousands of shore birds and diving ducks.

The Lower Suwannee NWR provides habitat for 13 threatened or endangered species, including bald eagle (*Haliaeetus leucocephalus*) and wood stork (*Mycteria americana*). Of particular interest in the river proper is the threatened Gulf sturgeon (*Acipenser oxyrhynchus desotoi*). Information about contaminant residues in Gulf sturgeon body tissues is scarce. The Service has evaluated six sturgeon from the Apalachicola River. These fish weighed between 4 and 108 pounds. The mercury range in the muscle tissue was 0.05 to 0.34 ppm wet weight. At present, no sturgeon mercury data are available for the Suwannee River.

trophic level fresh water predator fish, the analysis of muscle tissue from those fish, and an evaluation of the data as it related to age, length, and weight of the fish collected.

Investigations into the identification of specific mercury sources, the mechanisms of mercury transport and deposition, and the dynamics of mercury biotransformation and biomagnification within biota were beyond the scope of this study.

MERCURY

Mercury (Hg) and its compounds do not have any known normal metabolic function. The presence of mercury in cells of living organisms represents contamination from natural and/or anthropogenic sources. Any such contamination should be regarded as undesirable and potentially hazardous (Eisler 1987). Additional information about the nature of mercury is provided in Appendix A.

SELENIUM

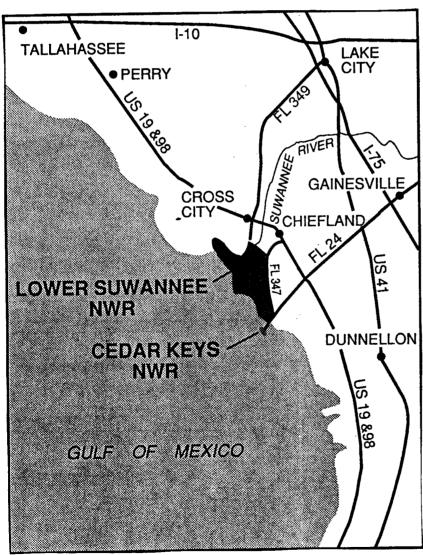
Selenium, a non-metallic element, occurs naturally in the environment in trace amounts and rarely exceeds 2 ppm dry weight in soils. Selenium is an essential micronutrient for normal animal nutrition, but concentrations exceeding those required may produce toxic effects ranging from physical malformations during embryonic development to sterility and death. Additional information about selenium is provided in Appendix B.

INTERACTIONS OF MERCURY AND SELENIUM IN BIOTA

Research indicates that selenium may provide protective action that lessens the toxic effects of mercury in many living organisms. The protective action of selenium against adverse or lethal effects induced by inorganic or organic mercury salts has been reported for algae, aquatic invertebrates, fish and mammals (Eisler 1985, 1987). Selenite salts can release methyl mercury from its linkage to proteins, and there is general agreement that a true antagonism exists between selenium and mercury, although the exact mechanism is not fully established (Eisler 1987). For example, in marine mammals and humans, selenium and mercury concentrations are closely related, almost linearly in a 1:1 molar ratio, but this relation blurs in teleost fishes (in which selenium is abundant) and fails in birds (Eisler 1985). Additional information about the protective action of selenium can be found in Appendix C.

Figure 1. Location of Lower Suwannee National Wildlife Refuge





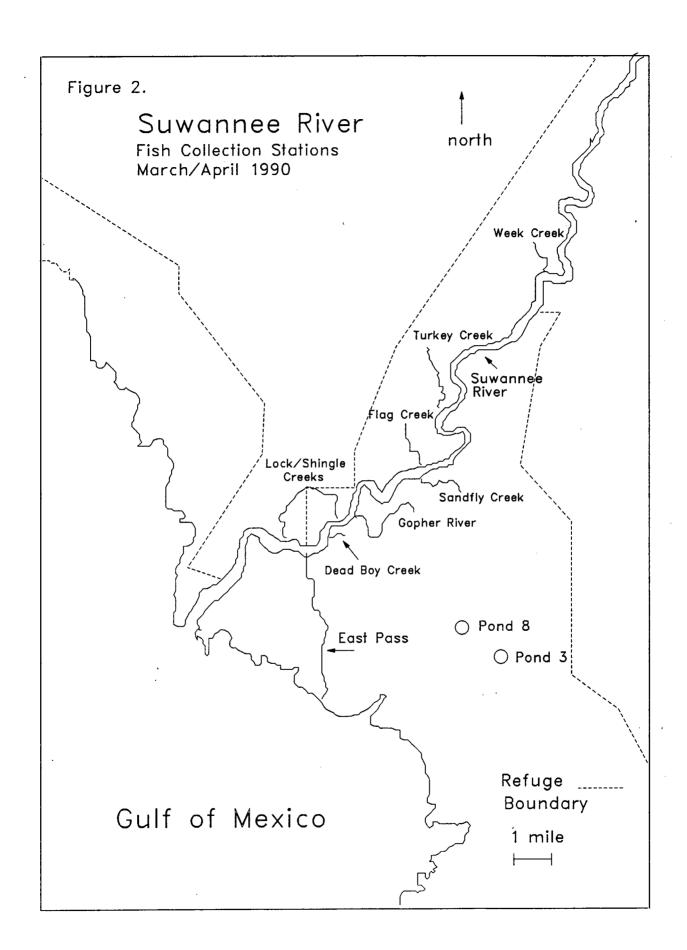
The Suwannee River is a receiving water for several major industrial discharges. A kraft mill located south of Valdosta, Georgia, discharges paper mill effluent into the Withlacoochee River approximately 95 river miles above the Refuge. Approximately 18 miles below the point of discharge, the Withlacoochee River flows into the Suwannee River at the location of the Suwannee River State Park. A power plant is located south of the State Park and discharges its cooling waters into the river. A phosphate mining operation is located at White City and discharges some amounts of mercury into Hunter and Swift creeks which both flow into the Suwannee River (Environmental Protection Agency, 1992).

SAMPLING STATIONS

For this study, sampling stations were defined as either entire ponds on Refuge lands or stretches of river or creeks large enough to allow fish collection by electrofishing, gill netting, or trotlining. River/creek sampling stations were usually approximately one mile or more in length. The sampling stations for the study (Figure 2) were primarily creeks and ponds within or adjacent to the Refuge. The sampling stations near the Refuge were selected because they receive drainage from Refuge lands, are desirable sites for recreational fishing, and because they are accessible from Refuge property as well as from the water. All streams sampled were slow flowing and tidally influenced. Most were surrounded by hardwoods and swamp forest vegetation, with adjacent uplands of pine flatwoods. Water draining from these flatwoods into the creeks contains tannic acid causing the water to be dark teacolored. None of the creeks were turbid or sediment-ladened.

A sampling station was also located about two miles above the mouth of the Santa Fe River. This sampling site is about 50 miles above the mouth of the Suwannee River. The Santa Fe River is a clear flowing, spring-fed system unaffected by tidal action. It is the largest tributary of the Suwannee River.

Two Refuge ponds (ponds 3 and 8) were sampled. One pond (pond 8) is a former borrow pit excavated in the early 1960s to obtain limerock to construct roadbeds. Both ponds are approximately one acre in size, and were stocked with largemouth bass in 1987 and 1988. The ponds have not been restocked since that time (Parauka, 1992).



MATERIALS AND METHODS

Fish were collected by electrofishing between March 22 and April 7, 1990. One sampling limitation was that the areas sampled had to be deep enough to accommodate the electrofishing boat including its outboard motor. Gill nets and trot lines were used in the small ponds. Collected specimens were immediately placed on ice in clean thermal containers and were taken back to the field trailer for sample preparation.

Fish samples were prepared within four hours of collection and stored in accordance with standard operating procedures for the collection of fish tissue samples (PCFO-EC-SOP-OO1, 1988), Appendix F. Otoliths were removed and sent to a Service contractor for age evaluation. Samples were frozen within eight hours of collection.

Upon returning to Panama City, samples were transferred to a storage freezer maintained at -23 degrees centigrade (-10 degrees Fahrenheit). Samples were shipped to the analytical laboratories after approximately 120 days of freezer storage. Laboratory protocols for handling and analysis of mercury and selenium are found in Appendix G. Appendix H contains the study data.

The fish mercury concentrations, morphometric measurements, and age data were statistically evaluated after the data for mercury and selenium concentrations were log-transformed to meet criteria for normal distribution. Statistically significant differences in mean mercury and selenium concentrations between locations were determined using single classification analysis at variance and Student-Newman-Keuls (SNK) procedure to measure differences among means (Sokal and Rohlf, 1969).

RESULTS

Table 1 presents the results of the field collections at the Lower Suwannee NWR and adjacent waters. Five species of fish were collected: largemouth bass (*Micropterus salmoides*), Suwannee bass (*Micropterus notius*), channel catfish (*Ictalurus punctatus*), yellow bullhead (*Ictalurus natalis*), and spotted gar (*Lepisosteus oculatus*). In the following sections, the data are evaluated for each species and each geographic sampling location.

MERCURY IN LARGEMOUTH BASS

Seventy-one largemouth bass were analyzed for mercury in muscle tissues (i.e., fish fillets). Fifty-five percent (n=39) had mercury exceeding the Florida lower-level consumption advisory of 0.5 ppm mercury, wet weight. Three percent (n=2) exceeded the upper-level consumption advisory of 1.5 ppm.

Table 1. Fish collections at locations in or adjacent to the Lower Suwannee NWR, 1990.

# Length: Weight: Mercury:°									
Sampling Location	Spp.	of Ind.	average (range)	average (range)	average (range)				
Lock/Shingle Creek	LMB	11	13°	20 ^b	.38 ^d .31°				
			(11-16)	(11-38)	(.14-1.2)				
Sand Fly Creek	LMB	8	13 (12-20)	25 (15-67)	.94 .82 (.29-1.75)				
Turkey Creek	LMB	3	14 (12-19)	32 (15-67)	.70 .69 (.5678)				
Dead Boy Creek	LMB	11	13 (12-14)	16 (12-22)	.39 .35 (.1995)				
Gopher River	LMB	6	13 (12-15)	19 (13-28)	.41 .39 (.1953)				
Flag Creek	LMB	1	13	16	.32				
Week Creek	LMB	13	13 (10-18)	17 (9-48)	.70 .65 (.2598)				
Suwannee River	LMB	5	15 (12-22)	31 (10-95)	.76 .75 (.59-1.01)				
	LMB- eggs	155 gms	na	na	.13				
Santa Fe River	LMB	13	14 (11-19)	25 (11-56)	.64 .59 (.25-1.0)				
	SB	4	12 (11-13)	16 (11-21)	.50 (.2765)				
Pond #3	YB SG	1 1	10 25	10 45	.98 2.41				
Pond #8	СС	1	19	45	.23				
	CC- eggs	222 gms	na	na	.01				
Species Codes: CC/channel catfish LMB/largemouth bass SB/Suwannee bass SG/spotted gar YB/yellow bullhead									

<sup>Length = inches
Weight = ounces
Parts per million, wet weight</sup>

^d arithmetic mean

[•] geometric mean

Mercury and Total Weight of Largemouth Bass

The largemouth bass data were sorted by Weight Groups (Figure 3). Weight Group I (1 lb or less) consisted of 38 fish. In this group, 47 percent (n=18) exceeded the 0.5 ppm concentration; however, only 1 fish exceeded 1.5 ppm.

Weight Group II (> 1 to 2 lbs) contained 24 individuals. Fifty-six percent (n = 13) exceeded the 0.5 ppm concentration. Only 1 fish exceeded 1.5 ppm.

Weight Group III (> 2 lbs) contained 9 fish. Eighty-nine percent (n = 8) exceeded the 0.5 ppm level. None in this small sample exceeded the 1.5 level.

There was a wide variation in mercury levels among fish of identical weight (Figure 4). Note particularly the variation among fish that weigh 16 ounces.

Mercury and Largemouth Bass Total Length

Largemouth bass total length was evaluated as a potential tool for roughly estimating, in the field, the amount of mercury that might be in an individual fish. The bass sample consisted of individuals ranging from 10 to 22 inches in length.

Small bass (12 inches or less; n=36) often exceeded the 0.5 ppm concern level (Figure 5). Seventeen of these fish (47 percent) exceeded the 0.5 ppm level, including one 10-inch fish. Two 12-inch fish exceeded the 1.5 ppm level.

Twenty-nine fish measured 13 through 16 inches in length. Fifty-five percent of these fish (n = 16) exceeded the 0.5 ppm level. None exceeded the 1.5 ppm level. However, we believe the sample sizes were too small to reveal the small percentage of individuals in each length category that would exceed 1.5 ppm. The fact that two individuals in the 12-inch category exceeded the upper level probably indicates that a small percentage of all length categories 12 inches or greater (in wild Suwannee River largemouth bass populations) would exceed 1.5 ppm.

All bass greater than 16 inches (n = 6) exceeded the 0.5 ppm level. The sample was too small to reveal information about the percentage of these larger fish in the wild that would regularly exceed the 1.5 ppm level.

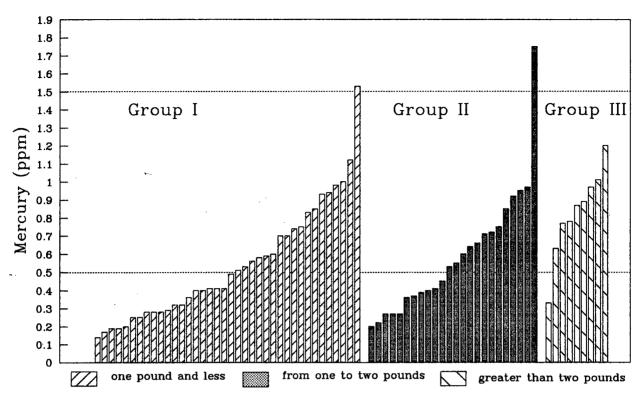


Figure 3. Variation of mercury concentration in largemouth bass by weight class, Lower Suwannee NWR, 1990.

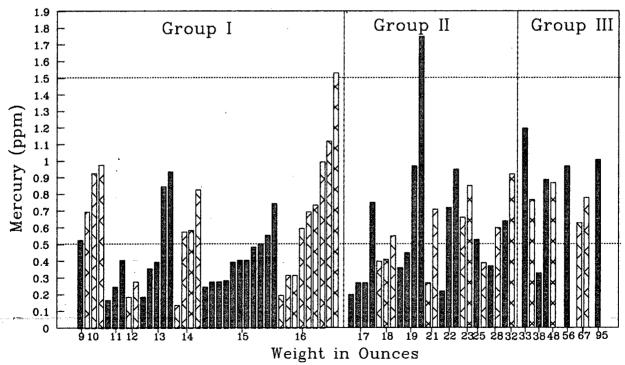


Figure 4. Variation of mercury concentration in individual largemouth bass by weight in ounces, Lower Suwannee NWR, 1990.

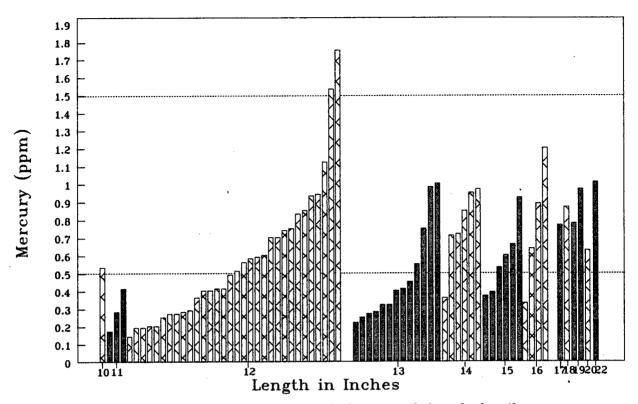


Figure 5. Variation of mercury concentration in largemouth bass by length, Lower Suwannee NWR, 1990.

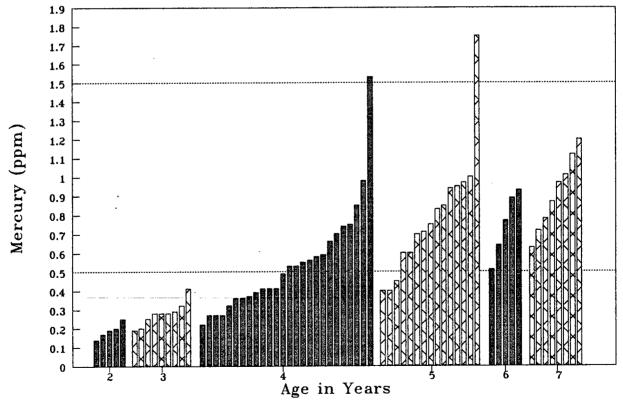


Figure 6. Variation of mercury concentration in largemouth bass by age, Lower Suwannee NWR, 1990.

Mercury and Age of Largemouth Bass

Generally, older bass had greater concentrations of mercury (Figure 6). Many of the bass collected that were four years old or older had mercury concentrations above 0.5 ppm, which may lead to the conclusion that bass must have exposure to the Suwannee River environment for at least four years before they concentrate mercury above the 0.5 ppm concern level. However, these data should be viewed with caution because the sample sizes of year class 2 and year class 3 bass were both so small (n=5) and (n=6), respectively).

SELENIUM IN LARGEMOUTH BASS

Because selenium may decrease the effects of mercury (Eisler, 1985, 1987), concentrations of selenium were measured in an attempt to understand possible relationships between it and mercury in largemouth bass. All but two bass had selenium concentrations between 0.1 and 1.0 ppm wet weight. The arithmetic mean concentration was 0.43 ppm (geometric mean 0.39; range 0.03-1.18).

In the most recent nationwide monitoring of selenium in freshwater fishes, selenium ranged from 0.05 to 2.9 ppm (wet weight, whole fish) and averaged about 0.6 ppm (Eisler 1985). In another study (Eisler 1987) selenium concentrations in muscle tissue of largemouth bass ranged from 0.05 to 1.7 ppm. Selenium concentrations of bass in this study appear to be within the normal range for the species.

Selenium and Total Weight of Largemouth Bass

Selenium concentrations for largemouth bass weight groups ranged from 0.03 to 1.18 ppm (Figure 7). Weight Group I (1 lb or less) consisted of 38 fish. Selenium concentrations ranged from a minimum of 0.15 ppm to a maximum of 0.81 ppm. Weight Group II (>1 to 2 lbs) contained 24 individuals. Selenium concentrations ranged from 0.17 ppm to 0.67. Weight Group III (>2 lbs) contained 9 fish. Selenium concentrations ranged from 0.03 to 1.18. Selenium concentrations varied widely even among fish of identical weight. Note particularly the 15-ounce and 16-ounce fish groups (Figure 8). Selenium, however, did not vary as greatly as did mercury.

Selenium and Total Length of Largemouth Bass

Selenium concentrations in bass muscle tissue were compared with total lengths (Figure 9). All fish of a particular length were grouped together. The bar graph illustrates that there was not a strong association between the length of a bass and

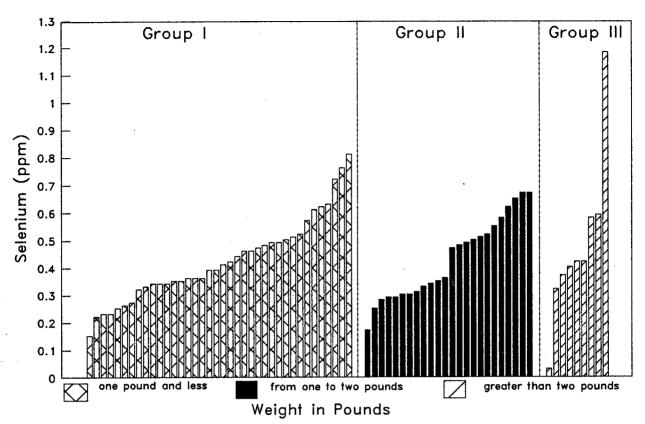


Figure 7. Variation of selenium concentration in largemouth bass by pound class Lower Suwannee NWR, 1990.

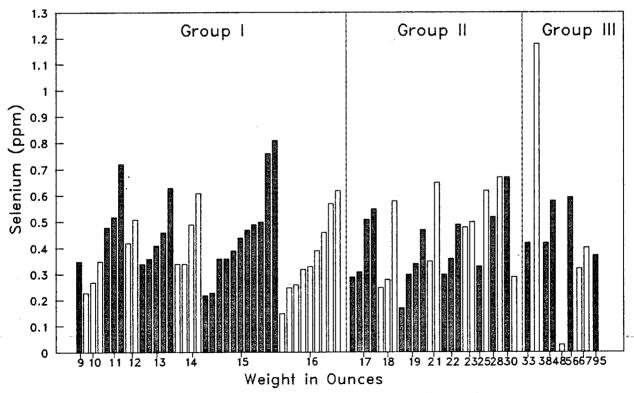


Figure 8. Variation of selenium concentration in individual largemouth bass by weight in ounces, Lower Suwannee NWR, 1990.

selenium in muscle tissue. Instead, a great deal of variability exists within each length group. Also, there are only minor differences in the ranges of selenium concentrations between groups of fish of different lengths. It is interesting, although probably coincidental, that the extremes in selenium concentrations observed are between a 17-inch individual with 1.18 ppm selenium, and an 18-inch individual with only 0.03 ppm selenium.

Selenium and Age of Largemouth Bass

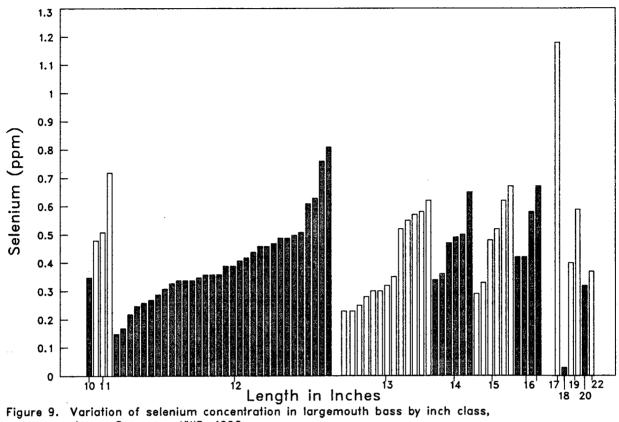
Selenium concentrations generally varied between about 0.2 ppm and 0.7 ppm for each age group, regardless of age (Figure 10). There is also considerable variation among individuals within any particular age group. The means for each age group are as follows (arithmetic mean/geometric mean):

AGE	2	3	4	5	6	7
Se	.54/.54	.45/.42	.39/.36	.46/.44	.62/.56	.39/.27

The extremes in selenium were the 17-inch fish (age 6; 1.18 ppm) and the 18-inch fish (age 7; 0.03 ppm). No strong association is apparent between age and selenium concentration in muscle tissue.

EVALUATION OF MERCURY AND SELENIUM RELATIONSHIPS IN LARGEMOUTH BASS

Average metal concentrations for each metal within the six age groups were compared (Table 2). Mercury appears to accumulate with length of environmental exposure (age); while selenium, an essential element, exhibited varied concentrations within a relatively narrow range.



Lower Suwannee NWR, 1990.

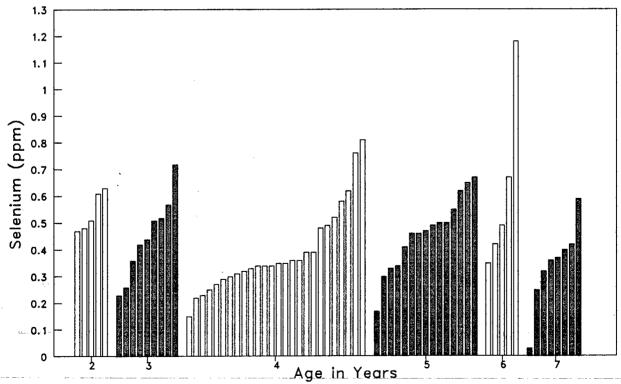


Figure 10. Variation of selenium concentration in largemouth bass by age class, Lower Suwannee NWR, 1990.

Table 2. Comparison of mean mercury and selenium concentrations between largemouth bass age groups (arithmetic mean/geometric mean). Concentrations are parts per million, wet weight.

Age Group	Number of Mean Individuals Hg Content		Mean Se Content
2	5	.19/.19	.54/.54
3	9	.28/.27	.45/.42
4	26	.54/.49	.39/.36
5	15	.79/.74	.46/.44
6	5	.75/.73	.62/.56
7	8	.91/.89	.39/.27

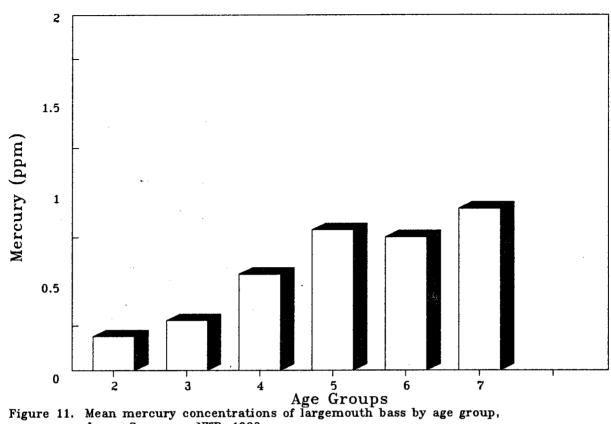
Figures 11 and 12 illustrate the age group differences in muscle tissue accumulation for mercury and selenium, respectively. Figures 13 and 14 reveal the distribution characteristics of these metals for all largemouth bass collected (n = 71).

EVALUATIONS BETWEEN LARGEMOUTH BASS SAMPLING LOCATIONS

Largemouth bass were collected at seven different locations (see Table 1). Several factors were evaluated at each location (Table 3), including: the percentage of each drainage basin located within the boundaries of the Refuge, the average ages of bass, and mean mercury and selenium concentrations of bass at each location. Collections from each location were evaluated for statistical differences. There were significant differences (P<0.05) between two locations near the Suwannee River mouth (Lock/Shingle Creek and Dead Boy Creek) and four other locations (Sand Fly Creek, Week Creek, Suwannee River, and Santa Fe River). All four of the latter had bass mean mercury concentrations significantly higher than Lock/Shingle and Dead Boy Creeks. However, the SNK statistical procedure did not detect significant differences in some cases that may indeed be significant. For example, at Sand Fly Creek, the mean mercury concentration was 0.94 ppm (geometric mean 0.82), while in Gopher River, immediately to the south, the mean concentration was 0.41 ppm (geometric mean 0.39). In this case, the SNK procedure detected no significant difference between those two locations. Probably our sample sizes (n = 8 and n = 6, respectively) limited the sensitivity of the statistical test. Therefore, further study of these two sampling locations may be warranted.

Four of the smaller backwaters have drainage basins entirely within the Refuge (Sand Fly, Dead Boy, and Flag Creeks; and Gopher River). Sand Fly Creek had the highest mean mercury of any collection location. Fish from this location were among the oldest fish collected. The mean mercury concentration in fish from Sand Fly Creek was twice as high as any other backwater site except Week Creek, in which the fish had only a somewhat lower mean mercury concentration.

Collection locations were also compared using only age-4 fish (Table 4). Sand Fly Creek, age-4 fish, had the same high concentration of mercury (nearly one part per million) as did bass of all ages collected in that creek. Age-4 fish at all other locations had either lower means or means that varied only slightly from the mean for bass of all ages. Week Creek has approximately half of its drainage basin on Refuge lands. The age-4 bass at Week Creek had a mean mercury concentration of 0.7 ppm. Both Sand Fly Creek and Week Creek may merit further field investigation.



Lower Suwannee NWR, 1990.

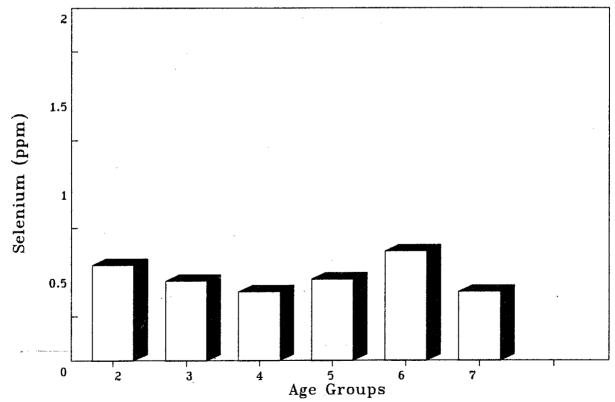


Figure 12. Mean selenium concentrations of largemouth bass by age group, Lower Suwannee NWR, 1990.

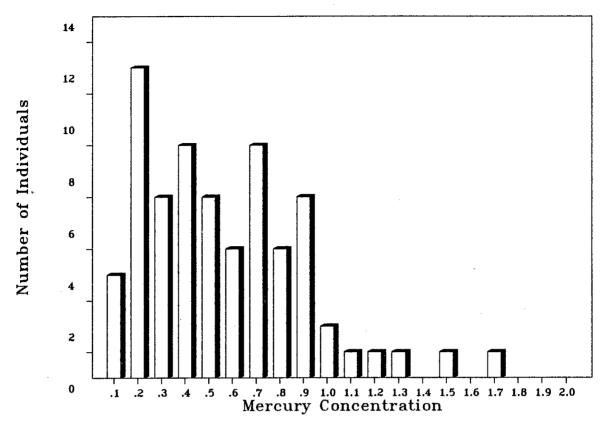


Figure 13. Mercury concentration in muscle tissue of largemouth bass, Lower Suwannee NWR and Santa Fe River, 1990.

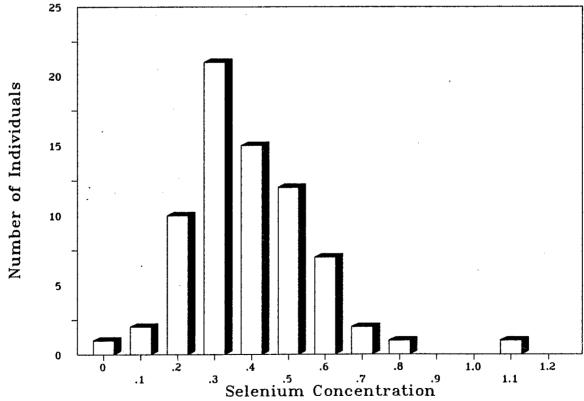


Figure 14. Selenium concentration in muscle tissue of largemouth bass, Lower Suwannee NWR and Santa Fe River, 1990.

Table 3. Age, and mean mercury and selenium content, of largemouth bass collected at the Lower Suwannee NWR. Also, the approximate percentage of each waterbody's drainage basin located inside the Refuge boundaries. Averages are arithmetic/geometric means.

Sampling Location	% on Rfg.	No. of bass	Avg Age	Avg* Hg	Avg* Se
Lock/Shingle	20%	11	4.00	.38/.31	.42/.40
Sand Fly Creek	100%	8	4.86	.94/.82	.28/.27
Dead Boy Creek	100%	11	3.55	.39/.35	.41/.40
Gopher River	100%	6	4.00	.41/.39	.42/.41
Week Creek	50%	13	4.69	.70/.65	.35/.30
Suwannee River	< 1%	5	4.60	.76/.75	.39/.38
Santa Fe River	0%	13	4.85	.64/.59	.65/.63
Turkey Creek	95%	3	5.00	.70/.69	.46/.41
Flag Creek	100%	1	3.00	.32/.32	.57/.57

^a Parts per million, wet weight

Table 4. Age, and mercury and selenium content, of age group 4 largemouth bass collected at the Lower Suwannee NWR. Also, the approximate percentage of each waterbody's drainage basin located inside the Refuge boundaries. Averages are arithmetic/geometric means.

Sampling Location	% on Rfg.	No. of bass	Avg Age	Avgª Hg	Avg* Se
Lock/Shingle	20%	4	4.00	.29/.28	.34/.34
Sand Fly Creek	100%	3	4.00	.94/.85	.28/.24
Dead Boy Creek	100%	6	4.00	.36/.35	.41/.37
Gopher River	100%	2	4.00	.45/.44	.42/.41
Week Creek	50%	3	4.00	.70/.67	.35/.30
Suwannee River	< 1%	4	4.00	.70/.69	.39/.38
Turkey Creek	95%	2	4.00	.65/.65	.49/.41
Flag Creek	100%	0	•	-	<u>-</u>

^a Parts per million, wet weight

OTHER FISH SPECIES

Spotted Gar: Mercury and Selenium in Muscle Tissue

One spotted gar (*Lepisosteus oculatus*) was captured in a gill net in Pond 3. The fish was 25 inches long and weighed just under three pounds (45 oz). The wet weight concentration of mercury in the muscle tissue was 2.41 ppm. This was the largest concentration of mercury found in any fish during this study. Unfortunately, the age of this fish is not known. The selenium concentration, at 0.14 ppm wet weight, was one of the lowest selenium values recorded during the study. Thus, this gar had the highest mercury level and one of the lowest selenium levels observed among all fish collected.

Yellow Bullhead: Mercury and Selenium in Muscle Tissue

One yellow bullhead (*Ameiurus natalis*) was also collected from Pond 3 on a trot line. The fish was ten inches long and weighed ten ounces. The mercury in this fish measured 0.98 ppm wet weight. Selenium was 0.08 ppm; the second lowest selenium level of any fish tested.

Channel Catfish: Mercury and Selenium in Muscle Tissue

One channel catfish (*Ictalurus punctatus*) was collected from Pond 8 on a trot line. The fish was a 19-inch female that weighed nearly three pounds (45 oz). The mercury in this fish measured 0.23 ppm wet weight. Selenium was 0.14 ppm. The fish was gravid, and eggs were collected and analyzed for mercury and selenium (see following section). This was the only fish collected from Pond 8.

FISH EGGS: MERCURY AND SELENIUM CONTENT

Samples of fully developed fish eggs were collected from one largemouth bass and one channel catfish. It appears that in the eggs of these fish, a consistent mercury/selenium relationship occurs. The fish eggs we collected, and fish eggs from other studies, had a high concentration of selenium compared to a low concentration of mercury. Table 5 provides data about this observation.

The degree to which the concentrations of mercury and selenium in fish eggs have a physiological or biochemical interrelationship is unclear. It is also unclear whether there is any biochemical interaction between mercury and selenium in the eggs of fishes.

Table 5. Comparison of mercury and selenium concentrations in fish eggs from the Lower Suwannee NWR and other collection locations.

Species	Collection Location	Hg (ppm)	Se (ppm)	Reference
largemouth bass	Suwannee River	0.13	1.32·	This study
channel catfish	Pond 8	0.01	1.01	
largemouth bass	Apalachicola River	0.04	0.94	Winger 1984
channel catfish	Apalachicola River	0.03	1.25	**
common carp	Tennessee	0.03	0.74	Huckabee 1974
striped bass	Flint River, GA	0.06	1.32	USFWS 1988
striped bass	Flint River, GA	0.15	0.97	99
Gulf sturgeon	Apalachicola River	0.04	1.32	97
common carp	Eleven Mile Creek	0.02	1.02	USFWS 1990
channel catfish	Eleven Mile Creek	0.003	1.60	91
gafftopsail catfish	Perdido Bay	0.22	1.22	11
gafftopsail catfish	Perdido Bay	0.39	1.60	98

DISCUSSION

Several factors were studied during our mercury investigation, including selenium concentration, fish total weight, fish total length, age of fish, and comparison between sampling locations.

The evaluation of fishes (primarily largemouth bass) collected from waters adjacent to, or on, lands of the Lower Suwannee NWR revealed that all specimens contained some mercury in muscle tissue; and that many contained concentrations ranging between the State advisory concentrations of 0.5 and 1.5 ppm wet weight.

Comparison between age and size showed that some bass were small, but were older specimens with relatively high concentrations of mercury. Individual bass that are poor competitors, or that reside in localized areas that are relatively unproductive may become stunted, but may accumulate mercury in higher concentrations merely because of the total length of environmental exposure. On the other hand, it appears that younger bass inhabiting areas with more mercury in the environment may accumulate excessive amounts in relatively short time periods.

For largemouth bass collected in this study, no clear relationship was observed between either total length or total weight of bass and amount of mercury in muscle tissue. Therefore, there appears to be no simple, convenient way for recreational fishermen or biologists to estimate the approximate amount of mercury in any individual bass based on these physical characteristics. Even if fishermen desire to keep small bass to avoid mercury, a recent bass management plan will require that bass caught in the Suwannee River be at least 12 inches in length (Florida Game and Fresh Water Fish Commission 1992). This minimum size limit is part of a new Florida largemouth bass management program that took affect on July 1, 1992.

Comparisons between concentrations of mercury and selenium in the fish collected indicate that mercury continues to increase in amount with time; whereas selenium, an essential element, maintains relatively constant concentrations.

The results of this study also suggest that some localized backwaters, particularly Sand Fly and Week Creeks, may provide an environment for fishes that results in higher tissue concentrations of mercury than in other backwater areas. Because all of the Sand Fly Creek drainage basin and about half of the Week Creek drainage basin are on Service lands, further investigation regarding mercury in these locations is probably warranted.

Our limited survey of Ponds 3 and 8 indicates minimal populations of fishes susceptible to our collection gear (gill nets and trot lines). Because of the low level of mercury in the one channel catfish taken from Pond 8 (a dredged borrow pit with little vegetation), this pond may provide a low mercury environment. On the other hand, Pond 3 and its associated drainage, appears to be an environment that encourages concentration of mercury in fish tissue. Logistics and field time did not allow surveys of other refuge ponds.

Although this study was limited primarily to the analysis of fish muscle tissue, other body tissues represent varying sources of mercury that are available to wildlife. The partitioning of mercury in other body compartments of fish is illustrated for two species in Table 6.

Table 6. Partitioning of mercury in some body compartments of two fish species. Fish were collected in St. Andrew Bay, Florida (spotted sea trout) Apalachicola River, Florida and Flint River, Georgia (striped bass). Mercury values are ppm wet weight. USFWS data, Panama City, Florida

Species	Muscle	Liver	Offal*	Fat	Gonad
Spotted Sea Trout	0.40	0.33	0.27	-	0.08
Spotted Sea Trout	0.56	0.28	0.26	-	0.10
Spotted Sea Trout	0.48	0.24	0.25	-	0.04
Striped Bass	0.45	0.37	_	0.06	0.06
Striped Bass	0.68	0.78	-	0.17	0.15
Striped Bass	0.40	0.24	-	0.02	-

^{*} Offal. The waste or byproduct of a process. In this case, the remainder of each fish after fillets (muscle tissue), the liver, mesentery fat, and the gonads had been removed.

The Table reveals that considerable amounts of mercury accumulate in the liver and other body parts. Not as much mercury accumulates in fat and reproductive tissue. Wildlife feeding on fish in the areas where our collections took place may be building up mercury in their body tissues. Species of particular concern include the bald eagle, wood stork, osprey, anhinga, and various species of fish-eating, wading birds.

CONCLUSIONS AND RECOMMENDATIONS

The mercury concentrations in fish tissue (edible fillet) from the study area often exceed State consumption advisory levels. Some waterbodies adjacent to the Refuge have significantly higher levels of mercury in the environment than others. At least one pond on the Refuge may also be an aquatic environment high in mercury. It is possible that certain species of migratory birds feeding in waters near the Refuge may be accumulating undesirable concentrations of mercury.

The following actions are recommended as a result of this study:

- 1) Further investigation of Sand Fly Creek and Week Creek environments and biota.
- 2) Limited sampling and chemical analysis of trust resource species (Gulf sturgeon, bald eagle, wood stork, osprey, and other migratory birds) and their food chain organisms.

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APPENDIX A

THE NATURE OF MERCURY

Mercury (Hg) and its compounds have no known normal metabolic function. The presence of mercury in cells of living organisms represents a contamination from natural and/or anthropogenic sources. Any such contamination should be regarded as undesirable and potentially hazardous (Eisler 1987).

Some forms of mercury with relatively low toxicity can be transformed into forms with very high toxicity through methylation and other biological processes. Methyl mercury can be bioconcentrated in organisms and biomagnified through food chains, returning mercury directly to man and other upper trophic level consumers in concentrated form. Mercury has mutagenic, teratogenic and carcinogenic properties, and has caused embryocidal, cytochemical and histopathological effects. High body burdens of mercury normally encountered in some species of fish and wildlife from remote locations emphasize the complexity of natural mercury cycles and human impact on these cycles. Some scientists believe that the anthropogenic release of mercury into the environment should be curtailed because the difference between tolerable natural background levels of mercury and harmful effects in the environment is exceptionally small (Eisler 1987).

Mercury from natural sources can enter the biosphere as a gas from terrestrial and oceanic volcanic activity, in solution or in particulate form. Cinnabar (HgS) is a common mineral in hot springs deposits and a major natural source of mercury. The global cycle of mercury involves degassing of the element from the earth's crust, evaporation from natural bodies of water, atmospheric transport (mainly in the form of mercury vapor), and deposition of mercury back onto land and water. Oceanic effluxes of mercury are tied to equatorial upwelling and phytoplankton activity and may significantly affect the global cycling of this metal. If volatilization of mercury is proportional to primary production in the world's oceans, oceanic phytoplankton activity represents about 36 percent of the yearly mercury flow to the atmosphere (Eisler 1987).

Human activities that contribute significantly to the global input of mercury include the combustion of fossil fuels, mining and reprocessing of gold, copper, and lead, operation of chloralkali plants, and disposal of batteries and fluorescent lamps. The production of electrical apparatus, industrial control instruments (switches, thermometers, and barometers, etc.), laboratory appliances, anti-fouling and mildew-proofing paints, chemical formulations to control fungal diseases of seeds, bulbs, and vegetables, dental amalgams, pulp and paper, pharmaceuticals, and metallurgy and mining, is contributing, or has contributed, mercury to the environment (Eisler 1987).

Mercury burdens in sediments and other non-biological materials are estimated to have increased up to five times prehuman levels; primarily as a result of man's activities. The estimated half-time residence value for mercury is comparatively short in the atmosphere, between 6 and 90 days, but is much longer in terrestrial soils, oceanic waters, and oceanic sediments where it is estimated to remain 1,000, 2,000 and more than one million years, respectively (Eisler 1987).

An elevated concentration of mercury (usually as methyl mercury) in any biological sample is often associated with proximity to human use of mercury. The elimination of mercury point-source discharges has usually been successful in improving environmental quality. However, elevated levels of mercury in biota may persist in contaminated areas long after the source of pollution has been discontinued. It is noteworthy that some groups of organisms with consistently elevated mercury residues may have acquired these concentrations as a result of natural processes, rather than from anthropogenic activities. These groups include older specimens of long-lived predatory fishes, marine mammals (especially seals and sea lions), and organisms living near natural mercury ore/cinnabar deposits.

Certain species of macrophytes strongly influence mercury cycling. For example, *Spartina alterniflora*, a dominant salt marsh plant in Georgia estuaries -- accounted for almost half the total mercury budget in that ecosystem (Eisler 1987). Mangrove vegetation plays a similarly important role in mercury cycling in the Florida everglades (Eisler 1987). These findings suggest that more research is needed on the role of higher plants in the mercury cycle. In aquatic ecosystems, removal of the source of anthropogenic mercury results in a slow decrease in the mercury content of sediments and biota. The rate of loss depends, in part, on the initial degree of contamination, the chemical form of the mercury and the half-life of that form, physical and chemical conditions of the system, and the hydro-dynamics of the particular aquatic ecosystem.

Methyl mercury is produced by methylation of inorganic mercury present in both freshwater and saltwater sediments, and accumulates in aquatic food chains in which the top level predators usually contain the highest concentrations (Eisler 1987). Most organomercury compounds other than methyl mercury decompose rapidly in the environment, and behave much like inorganic mercury compounds (Eisler 1987). In organisms near the top of the food chain, such as carnivorous fishes, almost all mercury accumulated is in the methylated form, primarily as a result of the consumption of prey containing methyl mercury. A strong relationship appears to exist between elevated mercury in Florida largemouth bass and low pH waters from swamp or peat drainage. A negative correlation exists in Florida for highly eutrophic waters (enriched), where depressed mercury levels are typically found.

Methylation also occurs within the biological organisms themselves because intestinal bacteria convert mercury into methyl mercury through enzymatic processes.

However, this methylation process, as a mercury uptake source, is not as important as intake of methyl mercury via the animal's diet.

There is no known effective antidote to counteract the effects of methyl mercury poisoning on the vertebrate central nervous system (Eisler 1987). Mercury binds strongly with sulfhydryl groups and has many potential target sites during embryogenesis. Phenyl mercury and methyl mercury compounds are among the strongest inhibitors of cell division (Eisler 1987). Organomercury compounds, especially methyl mercury, cross placental barriers and can enter mammals by way of the respiratory tract, gastrointestinal tract, skin or mucus membranes (Eisler 1987). Compared with inorganic mercury compounds, organomercurials are more completely absorbed, or more soluble in organic solvents and lipids, pass more readily across biological membranes, and are slower to be excreted (Eisler 1987).

Mercury, at comparatively low concentrations, adversely affects the reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation, and oxygen exchange of marine and freshwater organisms (Eisler 1987). In general, the accumulation of mercury by aquatic biota is rapid, and depuration is slow. Organomercury compounds, especially methyl mercury, have been found to be significantly more effective than inorganic mercury compounds in producing adverse effects and accumulations. Adverse affects of mercury to aquatic organisms have been documented at water concentrations of 0.88 to 5.0 ug/l. Enzyme disruption occurred in brook trout (Salvenlinus fontinalis) embryos exposed for 17 days in solutions containing 0.88 ug/l of methyl mercury (Eisler 1987). Increased incidence of frustule abnormalities and burst thecae were documented in two species of marine algae exposed to 1.0 ug/l concentrations of Hg++ for 24 hours (Eisler 1987). Arrested development of sea urchin larvae occurred in a 40-hour test when the larvae were exposed to 3.0 ug/l concentrations of Hg++ (Eisler 1987). Decreased rate of intestinal transport of glucose, fructose, glycine, and tryptophan occurred in the murrel, Channa punctatus, when exposed to 3.0 ug/l concentrations of Hg++ for 30 days (Eisler 1987). The blood chemistry of striped bass (Morone saxitalis) was altered when these fish were exposed to 5.0 ug/l concentrations of Hg++ for 60 days (Dawson 1982). Decreased respiration in striped bass was observed 30 days post exposure after immersion for 30 to 120 days in 5.0 ug/l concentrations of Hg++ (Eisler 1987).

The environmental cycle of mercury is delicately balanced and small changes in input rates, and/or the chemical forms of mercury, may result in increased methylation rates in sensitive systems. For example, the acidification of natural bodies of freshwater is statistically associated with elevated concentrations of methyl mercury in the edible tissues of predatory fishes. In chemically sensitive waterways such as poorly buffered lakes, the combined effects of acid precipitation and increased emissions of mercury to the atmosphere (with subsequent deposition) pose a serious threat to the biota if optimal biomethylation conditions are met.

APPENDIX B

THE NATURE OF SELENIUM

All investigators appear to agree on four points. First, that insufficient selenium in the diet may have harmful, and sometimes fatal, consequences. Second, that exposure to grossly elevated levels of selenium in the diet or water, is inevitably fatal over time to terrestrial and aquatic organisms. Third, that there is a comparatively narrow concentration range separating effects of selenium deficiency from those of selenosis. Fourth, that additional fundamental and basic research is required on selenium metabolism, physiology, recycling, interactions with other compounds or formulations, and chemical speciation in order to elucidate its nutritive role, as well as its toxic effects. Accordingly, the proposed selenium criteria for prevention of selenium deficiency and for protection of aquatic life, livestock, crops, and human health, should be viewed as guidelines, pending acquisition of additional, more definitive data.

Selenium chemistry is complex. In nature, selenium exists as six stable isotopes, three allotropic forms, and in five valence states.

Selenium, a non-metallic element, occurs naturally in the environment in trace amounts and rarely exceeds 2 ppm dry weight in soils. Selenium is an essential micronutrient for normal animal nutrition, but concentrations exceeding those required may produce toxic effects ranging from physical malformations during embryonic development to sterility and death. Two major sources of selenium are agricultural irrigation return-flows that originate from high selenium soils, and drainage water from areas used for storage and disposal of ash produced by coal-fired power plants.

Because selenium in aquatic systems is readily taken up by organisms, concentrations sometime reach levels toxic to fish and wildlife. Three things can happen to dissolved selenium when it enters an ecosystem: 1) it can be absorbed or ingested by organisms, 2) it can bind or complex with particulate matter, or 3) it can remain free in solution (Lemly and Smith 1987). Through deposition of biologically incorporated selenium and settling of particulate matter, selenium accumulates in the top layer of sediment and detritus. Biological, chemical, and physical processes move selenium out of, as well as into, the sediments. The sediments are only a temporary repository for selenium. Aquatic systems are dynamic and selenium can be cycled back into the biota and remain at elevated levels for years after waterborne inputs of selenium are stopped.

Selenium may be removed from solution and held in sediments through the natural processes of chemical and microbial reduction of the selenate form (Se VI) to the selenite form (Se IV) followed by adsorption onto clay and the organic carbon phase of particulates, reaction with iron species, and co-precipitation or settling.

Immobilization processes effectively remove selenium from the soluble pool, especially in slow-moving or still-water habitats and wetlands (Lemly and Smith 1987).

Selenium in sediments is particularly important to long-term habitat quality because mechanisms in aquatic systems can mobilize selenium into food chains, and thereby cause long-term dietary exposure of fish and wildlife when it is made available for biological uptake by oxidation and methylation processes.

The aquatic systems that accumulate selenium most efficiently are shallow, standing or slow-moving waters that have low flushing rates. Several of these habitat types often occur together in one aquatic system. Rivers may have fast-flowing waters, slow moving pools and standing-backwater areas, all within a few hundred meters. The degree of fish exposure to selenium varies among habitats according to intensity of use, type of use, and relative contributions of the various processes that regulate selenium cycling.

Selenium is chemically similar to sulphur and because it is an essential micronutrient, extensive bioaccumulation may result. Biomagnification of selenium (the accumulation of progressively higher concentrations by successive trophic levels of a food chain) usually ranges from 2 to 6 times between the producers (algae and plants) and the lower consumers (invertebrates and forage fish) (Eisler 1985). Top level consumers, such as predatory fish, may receive toxic selenium levels in the diet even though the concentration in water is low. The risk of toxicity through the detrital food pathway will continue despite a loss of selenium from the water column, as long as contaminated sediments are present.

Toxic effects of selenium fall into two categories: 1) mortality of juveniles and adults, and 2) reproductive effects (Lemly and Smith 1987). Complete reproductive failure can occur with little or no tissue pathology or mortality in the adult population. Field and laboratory data suggests that selenium at concentrations greater than 2-5 parts per billion (ppb) in water can be bioconcentrated in food chains and cause toxicity and reproductive failure in fish. Selenium may interact with several metals that can alter the expression of biological effects. Other factors such as temperature, nutrition disease, differences in species sensitivity, differences in the relative toxicity of the various chemical forms of selenium and other environmental stresses may affect the actual concentration of selenium that produces toxicosis.

APPENDIX C

INTERACTIONS OF MERCURY AND SELENIUM IN BIOTA

The protective action of selenium against the adverse or lethal effects induced by various metals and metalloids is well documented for a wide variety of plant and animal species; however, not all tests were conclusive. Studies with some species of freshwater teleost fishes demonstrated negligible antagonism of selenium against mercury (Eisler 1985).

Reasons to account for the antagonism of selenium and mercury (as well as other metals) include dietary source and chemical form of selenium, influence of sulfur, biological translocation of selenium or mercury to less critical body parts, and chemical linkage of selenium to mercury on a linear basis. The exact mode of interaction is probably complex and has not yet been resolved. In regard to diet, selenium of animal origin and in the form of selenate is less effective than selenium from plant and inorganic sources in preventing methylmercury neurotoxicity in experimental animals (Eisler 1985). Disruption of sulfur metabolism by selenium, the sulfur being replaced by seleno-amino acids and other cell constituents containing selenium in living organisms, is one probable cause of selenium poisoning. It is conceivable that selenium-mercury compounds formed within the organism would be sufficiently nonreactive biologically to interfere with sulfur kinetics, presumably -SH groups (Eisler 1985, 1987). Differential redistribution of selenium or mercury to less critical body parts may partly account for observed antagonisms. Pretreatment of marine minnows with selenium protects against mercury poisoning and causes a marked redistribution of mercury among organs, presumably to non-critical body parts, and this transfer may partly account for the observed selenium-mercury antagonisms in that species (Eisler 1985). Some investigators have reported that selenium results in increased mercury accumulations. Increased retention of mercury and other metals may lead to a higher level of biomagnification in the food chain and higher body burden in the individual, which might counteract the positive effect of decreased intoxication (Eisler 1987). Extensive research is under way on the chemical linkage of selenium and mercury (Eisler 1985).

APPENDIX D

Table D.1. Mercury and selenium concentrations in largemouth bass muscle tissue (n = 68). Fish are grouped by age and sex. Metal values are ppm wet weight. - = sex undetermined.

Age	<u>Sex</u>	<u>Hg</u>	<u>Se</u>	Hg/Se <u>Ratio</u>
2	- - - F	.14 .17 .20 .19 .25	.61 .48 .51 .63 .47	0.23 0.35 0.39 0.30 0.53
3	- - - - - - - - - - - - - - - - - -	.20 .28 .28 .19 .29 .32 .28 .25	.26 .51 .36 .42 .44 .57 .23 .52	0.77 0.55 0.78 0.45 0.66 0.56 1.22 0.48 0.57
4		.22 .41 .27 .27 .74 .36 .36 .27 .41 .32 .41 .53 .58	.30 .39 .31 .35 .39 .34 .36 .29 .58 .32 .36 .52 .33	0.73 1.05 0.87 0.77 1.90 1.06 1.00 0.93 0.71 1.00 1.14 0.71 1.61 1.70 1.51
4	F F	.55 1.53 .75	.25 .15 .22	2.20 10.20 3.41

Table D.1. cont'd.

				Hg/Se
<u>Age</u>	<u>Sex</u>	Ha	<u>Se</u>	Ratio
	F	.70	.27	2.59
	F	.66	.48	1.37
	F	.49	.81	0.60
4	M	.56	.76	0.74
	M	.85	.34	2.50
	M	.59	.49	0.20
	M	.98	.23	4.26
	. M	.39	.62	0.63
5	-	1.75	.17	10.29
	-	.95	.49	1.94
	-	.40	.46	0.87
	-	.60	.33	1.82
5	F	.85	.50	1.70
	F	1.00	.62	1.61
÷	F	.75	.55	1.36
	F	.71	.65	1.09
_	F	.60	.67	0.89
5	M	.70	.46	1.52
	M	.97	.47	2.06
	M	.45	.30	1.50
	M	.94	.41	2.29
	M	.83	.34	2.44
-	M	.40	.50	0.80
6	-	.51	.49	1.04
6	F	.93	.35	2.66
	F	.77	1.18	0.65
	F	.89	.42	2.12
6	M	.64	.67	0.96
7	· -	.72	.36	2.00
	-	1.20	.42	2.86
7	F	.78	.40	1.95
	F	.63	.32	1.97
	F	1.01	.37	2.73
	F	.97	.59	1.64
7	M	1.12	.25	4.48
	M	.87	.03	29.00

Table D.2. Largemouth Bass (sex undetermined); mercury and selenium concentrations in muscle tissue (n = 29). Fish are grouped by age. Metal values are ppm (mg/kg) wet weight.

<u>Age</u>	<u>Sex</u>	<u>Hg</u>	<u>Se</u>
2	-, -	.14 [.] .17	.61 .48
	_	.20	.51
	-	.19	.63
3	-	.20	.26
	-	.28	.51
	-	.28	.36
4	-	.22	.30
	-	.41	.39
	-	.27	.31
	-	.27	.35
		.74	.39
	-	.36	.34
	-	.36	.36
	-	.27	.29
	-	.41	.58
-	-	.32	.32
	-	.41	.36
	-	.37	.52
	-	.53	.33
	-	.58	.34
	-	.53	.35
5	· <u>-</u>	1.75	.17
•	-	.95	.49
	-	.40	.46
	-	.60	.33
6	-	.51	.49
7	. •	.72	.36
	-	1.20	.42

Table D.3. Female Largemouth Bass. Mercury and selenium concentrations in muscle tissue (n=25). Fish are grouped by age. Metal values are ppm (mg/kg) wet weight.

<u>Age</u>	<u>Sex</u>	<u>Hg</u>	<u>Se</u>
2 3	F F F F F F	.25 .19 .29 .32 .28 .25	.47 .42 .44 .57 .23 .52
4	F F F F	.55 1.53 .75 .70 .66 .49	.25 .15 .22 .27 .48 .81
5	F F F F	.85 1.00 .75 .71 .60	.50 .62 .55 .65
6	F F	.93 .77 .89	.35 1.18 .42
7	F	.78 .63 1.01 .97	.40 .32 .37

Table D.4 Male Largemouth Bass. Mercury and selenium concentrations in muscle tissue (n=14). Fish are grouped by age. Metal values are ppm (mg/kg) wet weight.

Age	<u>Sex</u>	<u>Hg</u>	<u>Se</u>
4	M	.56	.76
	M	.85	.34
	M	.59	.49
	M	.98	.23
	M	.39	.62
5	M	.70	.46
	M	.97	.47
	М	.45	.30
	M	.94	.41
	М	.83	.34
	M	.40	.50
6	M	.64	.67
7	М	1.12	.25
	M	.87	.03

APPENDIX E

LOWER SUWANNEE NATIONAL WILDLIFE REFUGE STUDY SITE LOCATIONS

Sample Number	Latitude/Longitude	Station Location
SWL-1 thru SWL-11	29°18′50"N/83°07′44"W 29°19′40"N/83°06′29"W	Lock/Shingle Creeks
SWM-1 thru SWM-2 SWM-21 thru SWM-26	29°20′21"N/83°04′40"W	Sand Fly Creek
SWM-3 SWM-28,29	29°21′32"N/83°04′14"W	Turkey Creek
SWM-4 thru 14	29°19′15"N/83°06′41"W	Dead Boy Creek
SWM-15 thru 20	29°19′49"N/83°06′07"W	Gopher Creek
SWM-27	29°20′36"N/83°04′36"W	Flag Creek
SWU-1 thru 4 SWU-10 thru 18	29°23′56"N/83°04′36"W	Week Creek
SU-5 thru 9	29°22′30"N/83°04′00"W 29°25′13"N/83°01′00"W	Suwannee River
SAF-1 thru 17	29°54′33"N/82°51′53"W 29°55′47"N/82°48′41"W	Santa Fe River
PD8-1 and PD8-1E	•	Pond #8
PD3-1 and PD3-2		Pond #3

STANDARD OPERATING PROCEDURES

COLLECTION OF FISH TISSUE SAMPLES

Fish collected for chemical contaminant evaluations may be taken by electrofishing gear, monofilament gillnets, otter trawl, haul or beach seines, fish traps, trotlines, or rod and reel. However, any collecting gear should be free of chemical treatments and/or metals that could contaminate samples. This is particularly important when the entire fish (whole body analysis) will be used.

For species of special concern such as Gulf sturgeon or large broodstock striped bass, we utilize only incidental mortalities, and these should be fresh specimens.

The following is a sample dissection.

- 1. Wash hands thoroughly and rinse completely. Wear vinyl or latex gloves. Final rinse with distilled water.
- 2. Fish should be clean. It may be rinsed of debris or mud in the waters of the collection site.
- 3. The dissection surface (work area) should be a chemically inert substance such as a stainless steel acetone-rinsed pan, or counter. Avoid letting the dissected sample touch this surface, if possible.
- 4. Use previously cleaned, and acetone rinsed, then distilled water-rinsed stainless steel dissection tools (knives, scalpels, etc.). Scales for total fish weights and sample weights should also be clean or covered with pre-cleaned aluminum foil. Measuring devices for fish lengths, etc., should be clean, or should not come in contact with the specimen.
- 5. Do not let dissected samples remain exposed to the air. Exposure can dry samples and reduce the natural percentage of moisture. Prepare each dissected sample for shipping or freezing as it is dissected.
- 6. Samples should be placed in the smallest, pre-cleaned glass jar that will adequately hold the sample. The jars should be pre-labeled with a permanent, waterproof marking pen on the outside of the jar. Jars should also have a teflon liner inside the lid. As an alternative, acetone-rinsed, heavy-duty aluminum foil may be used to wrap the sample. After double-wrapping, place the sample (with sample identification label) inside an air-tight zip-lock bag.

- 7. Sample identification labels should be prepared with permanent, waterproof ink or other writing instruments that will not bleed out or wash out, and should provide the following information:
 - a. species name and common name,
 - b. type of tissue (if not whole body),
 - c. collection location,
 - d. latitude and longitude,
 - e. county and state,
 - f. weight of sample in grams,
 - g. date of collection,
 - h. sample collector's name,
 - i. total weight of fish specimen (grams),
 - i. total length and fork length of specimen (cm), and
 - k. method of collection.
- 8. Samples should be frozen as soon as possible. If samples contain large amounts of liquids that may expand, the lids may be set on the jars, without securing, until the sample has expanded and frozen. Then lids should be secured tightly.
- 9. Photographs of the specimens are desirable, as well as a written description of any external or internal lesions, tumors, etc.

U. S. FISH AND WILDLIFE SERVICE PATUXENT ANALYTICAL CONTROL FACILITY

QUALITY ASSURANCE REPORT

RE: 6356

REGION: 4

REGIONAL ID: 90-4-106

THE ANALYSES ON THE ABOVE MENTIONED SAMPLES WERE PERFORMED AT:

THE ENVIRONMENTAL TRACE SUBSTANCES RESEARCH CENTER ROUTE 3 COLUMBIA, MISSOURI 65201

AFTER A THOROUGH REVIEW OF THE REPORTS ISSUED BY THE LABORATORY, I REPORT THE FOLLOWING OBSERVATIONS AND CONCLUSIONS:

THE ACCURACY, AS MEASURED BY SPIKE RECOVERY AND REFERENCE MATERIAL ANALYSIS, WAS ACCEPTABLE FOR ALL ANALYTES. AVERAGE RECOVERY FOR SPIKED SAMPLE ANALYSES IS GIVEN IN TABLE 1.

THE PRECISION, AS MEASURED BY DUPLICATE SAMPLE ANALYSIS, WAS ACCEPTABLE FOR ALL ANALYTES. AN ESTIMATE OF THE 95 % CONFIDENCE INTERVAL FOR THE METHODS USED IN THESE ANALYSES IS GIVEN IN TABLE 2.

QUALITY ASSURANCE OFFICER DATE

ANALYTICAL REPORT INTEGRITY FORM

CATALOG #: 6356 LAB: E15R REGIONAL ID: 90-4-106

DATE	INITIALS	PROBLEMS/ACTION
1-23-91	LW	INITIAL REVIEW OL
1-23-91	E(OA/OC Review - OR
4 .		
,		·



5450 South Sinclair Road Columbia, Missouri 65203 Telephone (314) 882-2151 Telefax (314) 882-3031

January 18, 1991

John Moore U.S. Department of the Interior Patuxent Wildlife Research Center Stickel Laboratory, Bldg 108 Laurel, Maryland 20708

Dear John:

Let me know if you have any questions.

Sincerely,

Edward J. Hinderberger, Jr.

Group Leader

EJH:ds

Enclosures



% MOISTURE

For animal tissue and sediments of sufficient size, moisture was determined by placing a weighed aliquot of the sample in a Fisher Isotemp oven and drying at 103-105°C. The dried sample was then weighed and the data entered into a computer program to generate the % moisture and final report.

Plants, and samples too small for oven dried moisture determination had the % moisture calculated from the moisture lost during the freeze-drying in the Labcono Freeze-Dryer 8. The data was entered into a computer program to generate a % moisture and final report.



HOMOGENIZATION

Large tissue samples, such as whole fish, were first run through a meat grinder one or more times depending on the size of the sample. An aliquot of the ground sample was weighed and frozen. For smaller tissue samples and plant samples the entire sample was weighed and then frozen. For sediments, the sample was mixed and an aliquot weighed and frozen. The frozen samples were placed in a Labcono Freeze Dryer 8 until the moisture had been removed. The dry samples were then weighed and further homogenized using a blender, or Spex Industries, Inc. Model 8000 mixer/mill with tungsten-carbide vial and balls.



MERCURY - COLD VAPOR ATOMIC ABSORPTION

Perkin-Elmer Model 403 AA Equipment used for Cold Vapor Atomic Absorption include: Perkin-Elmer Model 056 recorder; Technicon Sampler I; Technicon Pump II; a glass cell with quartz windows and capillary tube for entry and exit of the mercury vapor; and a liquid-gas separator. The samples were placed in 4 ml. sample cups at least 3/4 full. The samples were mixed with hydroxylamine for preliminary reduction, then stannous chloride for reduction to the mercury vapor. The vapor was separated from the liquid and passed through the cell mounted in the light path of the burner compartment. were recorded and the peak heights measured. The standardization was done with at least 5 standards in the range of 0 to 10 ppb. The correlation coefficient was usually 0.9999 or better and must have been at least 0.999 to have been acceptable. A standard was run every 8-10 samples to check for drift in the standardization. This was usually less than Standards were preserved with 10% v/v HNO₃, 1% v/v HCl and 0.05% w/v K₂Cr₂O₇. solution concentrations were calculated and the data entered into the AA calculation program which corrected for blank, dilution, sample weight, sample volume and entered the data into the LIMS system for report generation.



NITRIC REFLUX DIGESTION FOR MERCURY

Approximately 0.5 g. of sample was weighed into a freshly cleaned 50 ml. round bottom flask with 24/40 ground glass neck. For waters, 10 ml. of sample were measured into the flask. Five ml. of concentrated sub-boiled HNO $_3$ were added and the flask was placed under a 12 inch water-cooled condenser with water running through the condenser. The heat was turned up to allow the HNO $_3$ to reflux no more than 1/3 the height of the columns. Samples were allowed to reflux for two hours. Then the heat was turned off and the samples allowed to cool. The condensers were rinsed with 1% v/v HCl and the flasks removed. The samples were diluted with 1% v/v HCl in a 50 ml. volumetric flask and then transferred to clean, labeled, 2 oz. flint glass bottles.



NITRIC - PERCHOLORIC DIGESTION - (SELENIUM)

Approximately 0.5 g. of sample was weighed into a freshly cleaned 100 ml. quartz Kjeldahl flask. (Sediment samples and samples containing a high percent of silica were digested in 100 ml. teflon breakers.) For water samples, 50 ml. of sample were measured into a teflon beaker. Slowly 15 ml. of concentrated sub-boiled HNO₃ and 2.5 ml. of concentrated sub-boiled HClO₄ were added. Foaming may occur with some samples. If the foaming started to become excessive, the container was cooled in a beaker of cold water. After the initial reaction had subsided, the sample was placed on low heat until the evolution of dark red fumes had ceased. Gradually, the heat was increased until the HNO₃ began refluxing, samples were allowed to reflux overnight. (This decreased the chance for charring during the reaction with $HC10_4$.) After the refluxing, the heat was gradually increased until the HNO₃ had been driven off, and the reaction with $HC10_4$ had occured. When dense white fumes from the ${\rm HC10_4}$ were evident, the samples were removed from the heat and allowed to cool. Two ml. of concentrated sub-boiled HCl were added. flasks were replaced on the heat and warmed until the containers were hot to the touch or started to boil. They were removed from the heat, and 5-10 ml. of deionized water were added. Samples were allowed to cool. They were then diluted using deionized water in a 50 ml. volumetric flask and transferred to clean, labeled, 2 oz. polyethylene bottles.



ARSENIC AND SELENIUM BY HYDRIDE

The Varian VGA-76 hydride generation accessory was mounted on either a Perkin-Elmer Model 603 AA or Model 3030 (B) AA. Electrodeless Discharge lamps (EDL) were used. The instrument and EDL settings were taken from the instrument manuals. The burner mount fo a Perkin-Elmer Model 10 Hydride generator was modified slightly to hold the Varian quart. cell. The cell was aligned in the light path of the burner chamber and a very lean flamwas used for heating the cell. The two stock solutions were 50% v/v sub-boiled HCl and 0.6% NaBH, in 0.5% NaOH for Selenium and concentrated sub-boiled HCL and 1% NaBH, in 0.5% NaOH for Arsenic. Samples were diluted with 10% v/v sub-boiled HCl. Standards were prepared by dilution of Fisher 1000 ppm stock with 10% v/v sub-boiled HCl in the range of 0 to 20 PPB. The instrument was standardized to read directly in PPB using S1 = 5.00 and S2 = 20.00. After standardization, the standardization was checked by reading other standards such as 2.00, 10.00 and 15.00 PPB and an instrumental quality control sample with a known value. If the standards and quality control were acceptable, the detection limit was determined by reading the zero standard 10 times, and twice the standard deviation of the mean was used as the detection limit. Samples were analyzed by taking an integrated reading for 3 seconds after the plateau was reached for the sample. This occured approximately 45 seconds after the sample tube was placed in the sample. Standardization was checked every 8-15 samples and approximately 10% of the samples were checked by the method of additions to monitor matrix effects. Matrix effects were usually not significant with the VGA-76. The data was corrected for drift of the standard curve and entered into the AA calculation program. This program corrected for blank, dilution, sample weight, sample volume and recorded the data in the LIMS database for report generation.

Appendix H Lower Suwannee NWR - Mercury/Fish Study

90-4-106	Station	dds	Ttl	Tt1	111	Tt1	Smpl	Hg conc.	Hg conc.	Se conc.	e con	Sex	Age
501 + 00	Location	1 1 1	Lng	Lng	Ngt	Wgt	9/0	DW (ppm)	(mdd) MM	(mdd) MO	(mdd) MM		yrs.
			E	in.	a b	20	moist	`	mg/kg	mcg/g m	/bɔ		Otol.
1	The property of the	T.MB	347	13	651	22	76.8	6	•		6.3	٠.	4
34L-1		LMB	309	12	413	14	78.3	0.65	0.14		S	۰.	7
7-TM3	, 4	I.M.B	320	12	433	15	78.5	1.9	0.41	•	3	۰.	4
0 1 1 1 V		LMB	324	12	470	16	78	0.92	0.2	•	N	۰.	m
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	, a	LMB	324	12	490	17	77.7	1.2	7	1.4	ന	٠.	4
2 TH2	, a	I.MB	288	11	345	12	76.7	1.2	•		n	۰.	ო
SWI7		LMB	299	11	335	11	78.2	0.17	0.17	•	4	٠.	7
2 TAC	ه (LMB	362	14	635	22	80			•	ന	6-	7
0 - 1Mg		LMB	343	13	615	21	77.9	1.2	۲.	•	ന	6٠	4
CWT. 10		I.MB	607	16	945	33	78.9		_:	73	4	6-	٦
SWI-10		LMB	420	16	1090	38	79.2	1.6	0.33	2.8	0.58	Ŀ	na
SWM-1		LMB	323	12	545	19	78.1		۲.	•	~	۰.	ហ
SWM-2	Sand Fly Creek	LMB	310	12	480	16	78.3		۲.	1.8	ന	٠. ١	4.1
SWM-3		LMB	505	19	1925	29	80.1		۲.	2	4	Ēų	7
SWM-4		LMB	328	12	445	15	78.7	1.3	4	1.7	ന		ო •
SWM-5	Dead Bov	LMB	360	14	565	19	78.8			•	m		d' '
SWM-6		LMB	310	12	390	13			۲,		ന		₫,
SWM-7		LMB	326	12	485	. 11			~		~		4
W.M.W.		LMB	367	14	625	22			٠:		4		ın.
O THE		I.MB	319	12	495	17	78.6	0.94	ö		ţ		7
SWM-10		I.MB	338	13	529	18	80				വ		4
SWM-11		I,MB	340	13	462	16		1.4			ന		4
SWM-12	Dead Boy	I.M.B.	325	12	450	12				•	4		9
SWM-13		LMB	310	12	428	15	77.4		0.41	1.6	ന		4
SWN-14		I.W.	315	12	358	12					4	E4	m
SWM-15		LMB	398	15	795	28		1.79		•	ഗ		4
SWM-16		LMB	340	13	516	18				•	(A		ಕ ದ
SWM-17		LMB	305	12	395	13	79.1	6		•	4		n (
SWM-18		LMB	308	12	375	13			0.19	•	Θ		7 1
SWM-19		LMB	327	12	480	16		ω	ď.	٠	י ניי		n ·
SWM-20		LMB	384	15	710	25		4	-:	•	(')	1	d ·
SWM-21	-	LMB	340	13	537	18	79.2	2.65	~:	•	(A .	ابدة	d i (
SWM-22	Fly	LMB	328	12	452	15		_	•	N	a. e	4 F	י ני
SWM-23		LMB	400	12	911	32		_	•: •	•		u, G	ğ <
SWM-24	and Fly	LMB	315	12	462	9 :		-	•	•	٠,	4 >	* 1~
SWM-25	and Fly	LMB	323	12	455	16		4	:	•	4 "	4 5	٠,
SWM-26		LMB	515	20	1916	6.7			•	•	, ,	4 6	۰, ۳
SWH-27	Flag Creek	LMB	345	13	470	16	78.8	'nι		7.7	70.0	>	n <
SWM-28	Turkey Creek	LMB	313	12	435	12				٠,	٠,	5 5	* <
SWM-29		LMB	322	12	450	12			. (-4	•	4 >	, 14
SWU-1		LMB	315	12	470	16			S	•	•	E :	nш
SWU-2	Week's Creek	LMB	356	14	525	19			•	•	٠.	Σ >	n u
SWU-3		LMB	345	13	540	19			٠	•	-	Σļβ	nц
SWU-4		LMB	362	14	689	23			•		ຄຸ	u ?	ი •
SWU-5	Suwannee River	LMB	306	12	371	13		4	•	٠	'n,	Σ;	d ' •
SWU-6		LMB	321	12	400	14		2.8	Ø. 559	2.3	9.4.0	ξΙ	31 15
SWU-7	Suwannee River	LMB	574	22	2700	95		6.	•	•	m (iu I	, ;
SWU-7E		LMB Eggs		0	155	വ		•	٠	•	m.	ing.	n n

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0.7	7	σ.	σ.	4	æ	σ.	ω.	3	.5	4.	 1	۲.	۲.	φ.	σ.	9.	0.71	0	4	۳.	4	7	9.	ø	٠,	4	•	•	86.0	•
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77.9 78	78.8									_		-		_		-	79.8	_	-	-	-			•		79.3	79.4	8	83.4	•
10 23	15	10	10	11	48	13	14	14	თ	15	16	17	37	38	26	30	21	59	11	56	12	19	21	15	11	12	45	7	10	45
298	440	309	299	325	1385	390	402	397	276	445	465	487		1105	_	864	598	838	329	755	426	548	601	435	319	358	1300	222	311	1298
12 15	13	13	12	13	18	12	12	12	10	12	13	13	17	16	19	16	14	15	11	15	12	12	13	12	11	12	19	0	10	25
317 395	346	335	325	335	467	310	311	321	279	326	335	341	437	425	490	412	367	396	296	391	310	322	351	318	303	305	499	Ø	d 27	645
LMB	LMB	LHB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	LMB	SW.Bass	SW.Bass	LMB	SW.Bass	SW.Bass	Chan.Cat.	at.Eg	Yell.Bullhea	ed Ga
Suwannee River Suwannee River				Week's Creek		Week's Creek		Week's Creek	Week's Creek	Santa Fe River	anta Fe			Santa Fe River	E4 E4		anta Fe		e e	anta	anta Fe R	Santa Fe River	Ð	Santa Fe River	Rive	Santa Fe River			Pond #3	
SWU-8 SWU-9	SWU-10	SWU-11	SWU-12	SWU-13	SWU-14	SWU-15	SWU-16	SWU-17	SWU-18	SAF-1	SAF-2	SAF-3	SAE-4	SAF-5	SAF-6	SAF-7	SAF-8	SAE-9	SAF-10	SAF-11	SAF-12	SAF-13	SAF-14	SAF-15	SAF-16	SAF-17	PD8-1	PD8-1E	PD3-1	PD3-2

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